(2)

Institute Report No. 330

Acute Oral Toxicity of Physostigmine Salicylate in Guinea Pigs

Denzil F. Frost, MS, DVM, CPT, VC
and
Don W. Korte, Jr., PhD, MAJ, MSC

MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

November 1988

Toxicology Series: 217

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

AD-A202 783

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3 DISTRIBUTION / AVAILABILITY OF REPORT APPROVED FOR PUBLIC RELEASE; DISTRIBUTION IS UNLIMITED.
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Institute Report No.: 330	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Mammalian Toxicology Division of Toxicology 6b. OFFICE SYMBOL (If applicable) SGRD-ULE-T	7a. NAME OF MONITORING ORGANIZATION US Army Medical Research Institute of Chemical Defense
6c. ADDRESS (City, State, and ZIP Code) Letterman Army Institute of Research Presidio of San Francisco, CA 94129-6800	7b. ADDRESS (City, State, and ZIP Code) Aberdeen Proving Ground Maryland 21010-5425
8a. NAME OF FUNDING / SPONSORING ORGANIZATION US Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21701-5012	10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO: 62734 / PROJECT NO: A875 / TASK NO: BC / WORK UNIT ACCESSION NO: DA0H0366

11. TITLE (Include Security Classification)
(U) Acute Oral Toxicity of Physostigmine Salicylate in Guinea Pigs

12. PERSONAL AUTHOR(S)
DF Frost and DW Korte, Jr.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Institute	FROM 7JUL87 TO 24SEP87	November 1988	63

16. SUPPLEMENTARY NOTATION
Toxicology Series No. 217

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Physostigmine Salicylate, Acute Oral Toxicity, Guinea Pig, RA V

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The acute oral toxicity of physostigmine salicylate was determined in male and female Hartley guinea pigs using the single-dose method. The median lethal dose for both male and female guinea pigs was less than 7.1 mg/kg. Clinical signs observed were primarily related to changes in behavior; such as tremors, hypertonia, irritability, ataxia, and inactivity. Other frequently observed clinical signs included salivation, diarrhea, and lacrimation. The duration of the clinical signs was acute. Most animals were exhibiting signs by 24 hours after dosing and had either died or returned to normal by 72 hours after dosing. According to the classification scheme of Hodge and Sterner, these results place physostigmine salicylate in the highly toxic class. Keywords:

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	UNCLASSIFIED

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
EDWIN S. BEATRICE, COL, MC	(415) 561-3600	SGRD-ULZ

DD Form 1473, JUN 86 Previous editions are obsolete. SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED

ABSTRACT

The acute oral toxicity of physostigmine salicylate was determined in male and female Hartley guinea pigs using the single-dose method. The median lethal dose for both male and female guinea pigs was less than 7.1 mg/kg. Clinical signs observed were primarily related to changes in behavior; such as tremors, irritability, ataxia, and inactivity. Other frequently observed clinical signs included salivation, diarrhea, and lacrimation. The duration of the clinical signs was acute. Most animals were exhibiting signs by 24 hours after dosing and had either died or returned to normal by 72 hours after dosing. According to the classification scheme of Hodge and Sterner, these results place physostigmine salicylate in the highly toxic class.

Key Words: Acute Oral Toxicity, Physostigmine Salicylate, Guinea Pig, RA V

PREFACE

TYPE REPORT: Acute Oral Toxicity GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Medical Research Institute of Chemical Defense
Aberdeen Proving Ground, MD 21010-5425
Project Officer: LTC J. von Bredow, PhD, MSC

PROJECT/WORK UNIT/APC: 3M162734A875/308/TLE0

GLP STUDY NUMBER: 87008

STUDY DIRECTOR: Don W. Korte, Jr., PhD, MAJ, MS

PRINCIPAL INVESTIGATOR: Denzil F. Frost, MS, DVM, CPT, VC
Diplomate, American College of
Veterinary Preventive Medicine

PATHOLOGIST: Charles B. Clifford, DVM, PhD, MAJ, VC
Diplomate, American College of Veterinary
Pathologists

DATA MANAGER: Yvonne C. LeTellier, BS

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocols, retired SOPs, raw data, analytical, stability, and purity data of the test compound, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Physostigmine Salicylate

INCLUSIVE STUDY DATES: 7 July - 24 September 1987

OBJECTIVE: The objective of this study was to determine the acute oral toxicity of physostigmine salicylate in male and female Hartley guinea pigs.

ACKNOWLEDGMENTS

SPC Dean Magnuson, BS, and SPC Joel B. Seewald, BS, provided research assistance; SGT John R. G. Ryabik, BS, provided chemical preparation and analysis; SGT Tammie R. Heineman, SGT Chuck Freedman, and Richard A. Spieler provided animal care and facility management; and Marie Rogers and Mara Joshua provided secretarial assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, declare that study number 87008 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte 1 Dec 88
DON W. KORTE, JR, PhD/DATE
MAJ, MS
Study Director

Conrad R. Wheeler 1 Dec 88
CONRAD R. WHEELER, PhD/DATE
DAC
Analytical Chemist

Denzil F. Frost 1 Dec 88
DENZIL F. FROST, MS, DVM/DATE
CPT, VC
Principal Investigator

Yvonne C. LeTellier 1 Dec 88
YVONNE C. LeTELLIER, BS/DATE
DAC
Data Manager

Charles B. Clifford 1 Dec 88
CHARLES B. CLIFFORD, PhD, DVM/DATE
MAJ, VC
Pathologist

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF

SGRD-ULZ-QA

30 November 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 87008

1. This is to certify that in relation to LAIR GLP Study 87008, the following inspections were made:

06 April 1987	-	Protocol Review
23 July 1987	-	Dosing and Observations (Phase I)
04 August 1987	-	Final Sacrifice (Phase I)
08 September 1987	-	Dosing (Phase II)
08 September 1987	-	Observations (Phase II)
22 September 1987	-	Final Observations (Phase II)
22 September 1987	-	Final Sacrifice (Phase II)

2. The institute report entitled "Acute Oral Toxicity of Physostigmine Salicylate in Guinea Pigs," Toxicology Series 217, was audited on 14 November 1988.

Carolyn M. Lewis

CAROLYN M. LEWIS
Chief, Quality Assurance

TABLE OF CONTENTS

TABLE OF CONTENTS (cont.)

Acute Oral Toxicity of Physostigmine Salicylate in Guinea Pigs -- Frost and Korte

INTRODUCTION

Soman, the primary nerve agent utilized by threat forces, is refractory to the standard antidotal therapy, atropine and pralidoxime (2-PAM), fielded by the US Army. Consequently, the highest priority has been placed on fielding a more effective treatment regimen. A regimen incorporating pyridostigmine as a prophylactic agent, combined with standard atropine/2-PAM therapy, has proven extremely effective in reducing mortality of Rhesus monkeys to multilethal concentrations of soman (1). However, these animals require a prolonged period of recovery during which they are completely incapacitated. This has been attributed to the quaternary nature of pyridostigmine, which does not cross the blood-brain barrier and thus only protects the peripheral nervous system. Consequently, a tertiary carbamate, physostigmine, has been proposed for the prophylactic regimen since it would protect the central nervous system in addition to the peripheral nervous system. Experimental studies support this hypothesis as animals pretreated with physostigmine before exposure to soman recover at a faster rate than animals pretreated with pyridostigmine (2,3). An enhanced rate of recovery of soldiers from a multilethal exposure to soman would produce a decided advantage in maintaining a fully functional military unit during a future conflict.

The only approved formulation of physostigmine is for intravenous administration, which is not a feasible option for the proposed prophylactic therapy. Either the oral or dermal route of administration for prophylactic therapy would be feasible. However, even though physostigmine has been available for more than a century (4), little directed research on its toxicology following oral or dermal administration has been conducted. Consequently, the Division of Toxicology, Letterman Army Institute of Research, was tasked by the US Army Medical Research Institute of Chemical Defense to provide an acute and subchronic toxicity profile of physostigmine salicylate following oral and subcutaneous administration.

Objective of Study

The objective of this study was to determine the acute oral toxicity of physostigmine salicylate in male and female Hartley guinea pigs.

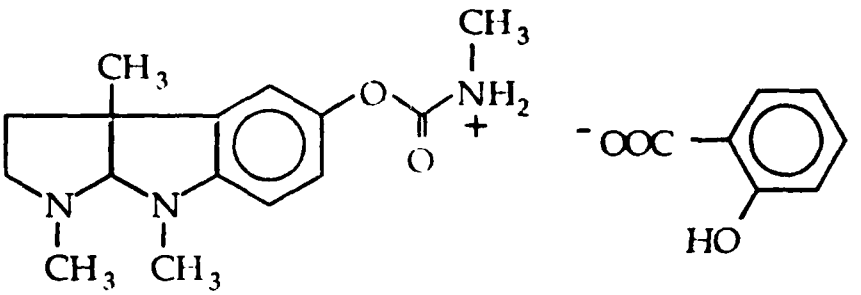
MATERIALS

Test Substance

Chemical Name: Physostigmine salicylate

Chemical Abstracts Service Registry No.: 57-64-7

Chemical Structure:



Molecular Formula: $C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$

Source: Mr. William Ellis
Division of Experimental Therapeutics
Walter Reed Army Institute of Research
Requested by LTC J. von Bredow, USAMRICD

Other test substance information is presented in Appendix A.

Vehicle

The vehicle for physostigmine salicylate was sterile water (Abbott Labs, North Chicago, IL 60064). The expiration date was 1 February 1989, and the lot number was 01-075-FW.

Animal Data

Forty-seven male and 87 female Hartley guinea pigs (Charles River Laboratories, Inc., Kingston, NY) were used for this study. They were identified individually with ear tags. Two additional males and three females from the shipment were

randomly selected for quality control necropsy. The animal weights on receipt (8 Jul 87, 28 Aug 87) ranged from 188 g to 265 g. Additional animal data appear in Appendix B.

Husbandry

Guinea pigs were caged individually in stainless steel wire-mesh cages with automatic flushing dumptanks. No bedding was used in any of the cages. The diet, fed *ad libitum*, consisted of Certified Purina Guinea Pig Chow® Diet 5026 (Ralston Purina Company, St. Louis, MO); water was provided by continuous drip from a central line. The animal room temperature was maintained in a range from 17.7°C to 25.6°C with a relative humidity range of 38% to 67%. The photoperiod was 12 hours of light per day.

METHODS

Group Assignment/Acclimation

Study animals were randomized into 5 dose groups of 8 males each, 4 dose groups of 8 females each, 3 dose groups of 16 females each, and vehicle control groups of 5 males and 5 females each. Allocation was accomplished using a computer-based, stratified, weight-biased method. The Beckman TOXSYS® Animal Allocation Program was used in conjunction with a Beckman TOXSYS® Data Collection Terminal. The animals were acclimated for 12-19 days before the day of dosing. During this period they were observed daily for signs of illness.

Dosage Levels

The ALD determination indicated that the median lethal dose (MLD) was between 5 and 7.5 mg/kg. Based on these data, test dosages were selected (Table 1).

Compound Preparation

Specific concentrations for dosing were prepared in sterile water for injection (Abbott Laboratories, North Chicago, IL 60064, Lot No. 01-075-FW).

TABLE 1: Physostigmine Salicylate Dosages

Group	Dosage Level (mg/kg)
Phase I (males and females)	
1	5.62
2	6.31
3	6.65
4	7.08
5	7.50
6 (vehicle control)	-
Phase II (females only)	
1	5.62
3	6.65
5	7.50
7	4.47
8	8.91

Chemical Analysis of Dosing Solution

The concentration of physostigmine salicylate in the dosing solutions was determined by UV spectrophotometry (Appendix A). Actual concentrations of physostigmine salicylate in the dosing solutions ranged from 96.8% to 99.6% of the target concentration.

Test Procedures

This study was conducted in accordance with LAIR SOP OP-STX-36 (5).

The volume of dosing solution each animal received was based upon the desired dose level, the compound's concentration in suspension, and the animal's weight. Volumes ranged from 2.1 ml to 3.5 ml in the males and from 2.4 ml to 3.8 ml in females. The vehicle control group was given 2.7 ml to 3.3 ml sterile water. The volumes given were based on 10 ml/kg. Dosing was performed using the oral gavage method without animal sedation or anesthesia. Sterile disposable syringes (Becton, Dickerson & Co, Rutherford, NJ) fitted with 5-French feeding tubes (Seamless Hospital Products Co., Division of Dart Industries, Inc., P.O. Box 828, Wallingford, CN) were used for dosing. Phase I animals were dosed between 0745 and 1336 hours on 21 Jul 87 and 0737 and 0903 on 23 Jul 87; Phase II animals were dosed between 0943 and 1020 hours on 8 Sep 87, 0921 and 0939 on 10 Sep 87, and 0900 and 0919 on 15 Sep 87.

Observations

Observations for mortality and signs of acute toxicity were performed daily according to the following procedure: (a) animals were observed undisturbed in their cages; (b) animals were removed from their cages and given a physical examination; and (c) animals were observed after being returned to their cages. On the day of the dosing, the animals were checked intermittently throughout the day. Recorded observations were performed 1, 2, and 4 hours after dosing, and daily for the remainder of the 2 week test period. A second "walk-through" observation was performed daily and only significant observations recorded. Body weights were recorded once weekly during the course of the study.

Necropsy

Animals that died during the observation period were submitted for a complete gross necropsy. Those which survived the 14-day study period were submitted for necropsy immediately after sacrifice by barbiturate overdose.

Statistical Analysis

Statistical analyses were performed on the study results. The LD10, LD50, and LD90 were derived by the maximum likelihood method of probit analysis, as described by Finney (6). The program, PROBIT, developed for the Data General Computer, Model MV8000, was used to plot the probit curve and lethal dose values.

Duration of Study

Appendix C is a complete listing of historical events.

Changes/Deviations

The dosing phase of this study was accomplished according to the protocol and applicable amendments with the following exceptions: The dosage levels for Phase I and II were differentiated between males and females to allow a more accurate MLD determination for each sex, and pediatric feeding tubes were used in place of metal dosing needles to minimize potential trauma to the oral and esophageal mucosa. It is believed that none of these changes had any adverse effect on the results of this study.

Storage of Raw Data and Final Report

A copy of the final report, study protocol, raw data, retired SOPs and an aliquot of the test compound will be retained in the Letterman Army Institute of Research Archives.

RESULTS

Mortality

Sixty-three animals died as a result of the dosing. Fifty-one (81%) deaths occurred within 12 hours of dosing. An additional 11 (17%) deaths occurred by 48 hours after dosing. Table 2 lists the compound-related deaths by group and the percent mortality. Appendix D is a tabular presentation of cumulative mortality.

Lethal Dose Calculations

Lethal dose values were calculated by probit analysis, and the equation for the probit regression line was: $Y = -0.47 + 6.44 \log X$ (males) and $Y = 0.66 + 5.61 \log X$ (females), where X is the dose and Y the corresponding probit value. Lethal doses calculated from the equation for the probit regression line are presented in Table 3. Figures 1 and 2 graphically present the actual data points and the regression line.

TABLE 2: Compound-Related Deaths by Group

Group	Dose Level (mg/kg)	Compound Related Death/ Number in Group	Percent Mortality
		MALES	
1	5.62	3/ 8	38
2	6.31	1/ 8	13
3	6.65	4/ 8	50
4	7.08	4/ 8	50
5	7.50	5/ 8	63
6	Control	0/ 5	0
		FEMALES	
7	4.47	2/ 8	25
1	5.62	8/16	50
2	6.31	5/ 8	63
3	6.65	9/16	56
4	7.08	5/ 8	63
5	7.50	9/15	60
8	8.91	8/ 8	100
6	Control	0/ 5	0

Clinical Observations

Fifty-one out of 129 dosed animals died within 12 hours of dosing. Clinical signs, in many cases, were observed before death. Many of the surviving 79 dosed animals exhibited clinical signs within the first 24 hours after dosing. The clinical observations included gastrointestinal, behavioral, reflexive, skin/fur, urogenital, and ocular signs.

TABLE 3: Calculated Lethal Doses (LD) of Physostigmine Salicylate in Sprague-Dawley Rats

Level	Calculated Dose* (mg/kg)
MALES	
LD10	4.48 ± 1.35
LD50	7.08 ± 0.62
LD90	22.0 ± 2.57
FEMALES	
LD10	3.51 ± 0.78
LD50	5.94 ± 0.40
LD90	10.05 ± 1.66

* Calculated dose ± standard error

The most frequently observed category of signs was gastrointestinal disturbances (72 of 129 animals). Gastrointestinal signs exhibited by the animals included salivation, diarrhea, tarry feces, stain or material found on animal, and emaciation. Gastrointestinal signs were present in all dose groups but did not exhibit a dose-response relationship. Other classes of clinical signs observed and their frequencies included: behavioral (15/129), reflexive (1/129), skin/fur (7/129), ocular (1/129), and urogenital (1/129). Of interest was one prolapsed uterus 2 days after dosing (animal 87E002e1).

Most clinical signs had cleared by 72 hours after dosing, with the exception of diarrhea, inactivity, staining, pallor, tarry feces, rough coat, and emaciation. Twenty-five animals died within the first 48 hours without clinical signs being detected. Additional deaths with clinical signs observed occurred up to 4 days after dosing. Tables 4 and 5 contain a summary of clinical observations. Appendix E contains individual animal histories.

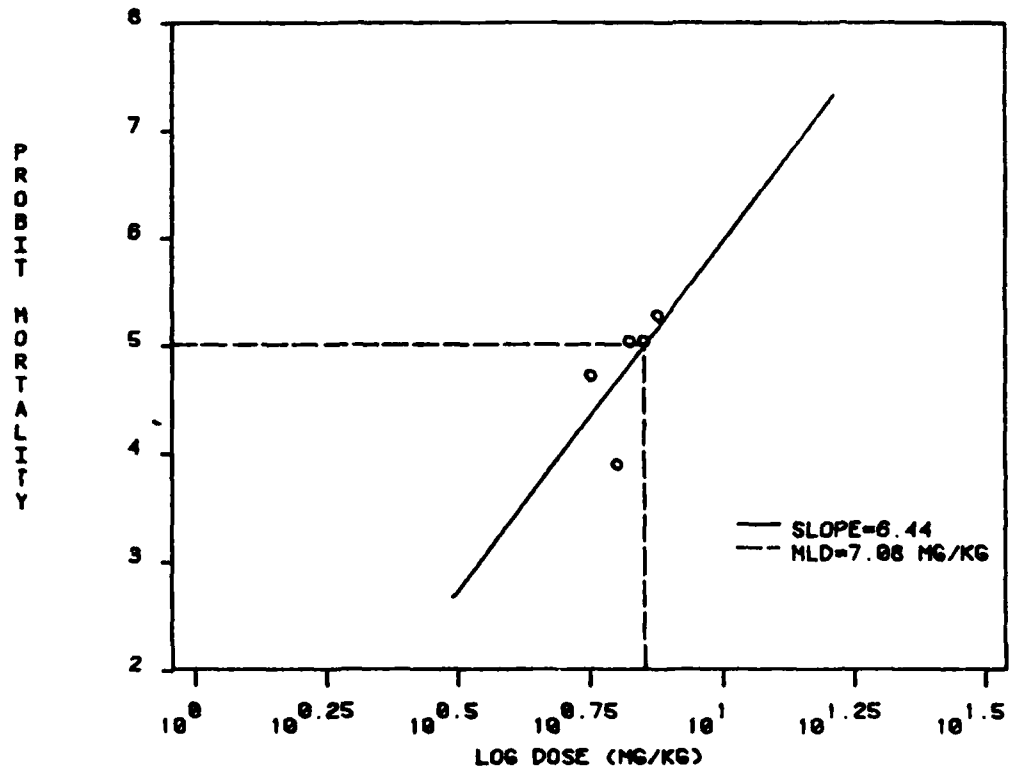


Figure 1: Physostigmine Dose Response Curve in Male Guinea Pigs

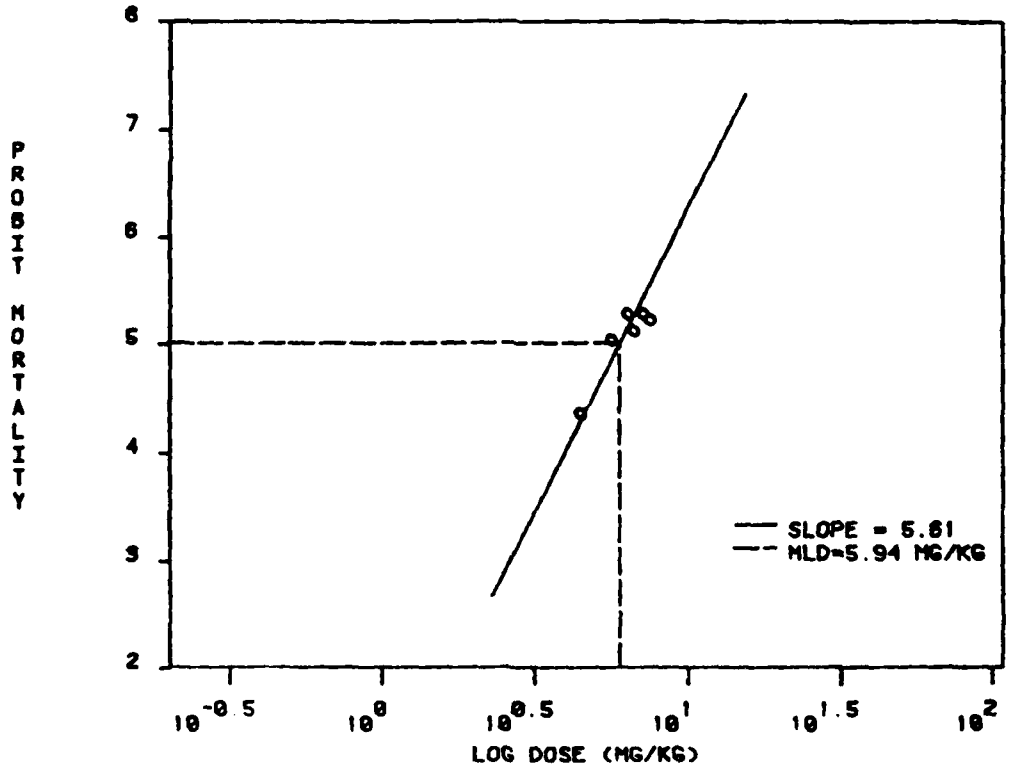


Figure 2: Physostigmine Dose Response Curve in Female Guinea Pigs

TABLE 4

Incidence Summary for Clinical Observations in Male Guinea Pigs
Administered Physostigmine Salicylate

Clinical Signs	Dose (mg/kg) (n=)	5.62 8	6.31 8	6.65 8	7.08 8	7.50 8	Vehicle 5
Behavior[a]		-	1	-	-	3	-
Gastrointestinal[b]		5	5	3	3	3	1
Skin/fur[c]		-	1	-	-	1	-

[a]Includes inactive and tremors.
[b]Includes increased salivation, diarrhea, tarry feces, stains or material found on animal, and emaciation.
[c]Includes rough coat.

TABLE 5

Incidence Summary for Clinical Observations in Female Guinea Pigs
Administered Physostigmine Salicylate

Clinical Signs	Dose (mg/kg) (n=)	4.47 8	5.62 16	6.31 8	6.65 16	7.08 8	7.50 15	8.91 8	Vehicle 5
General[a]		-	-	-	1	-	-	-	-
Behavior[b]		1	-	1	2	-	6	1	-
Reflexes[c]		1	-	-	-	-	-	-	-
Ocular[d]		-	-	-	-	-	1	-	-
Gastrointestinal[e]		7	9	5	9	5	9	6	2
Skin/fur[f]		2	1	1	2	-	-	-	-
Urogenital[g]		-	1	-	-	-	-	-	-

[a]Includes hunched posture.
[b]Includes inactive, ataxia, paralysis, and tremors.
[c]Includes loss of equilibrium.
[d]Includes lacrimation.
[e]Includes increased salivation, diarrhea, tarry feces, stains or material found on animal, and emaciation.
[f]Includes pallor and rough coat.
[g]Includes prolapsed uterus.

Weight gains of survivors were not significantly affected by dosing. Tables 6(a) and 6(b) present the mean body weights by groups. Appendix F contains individual weight tables.

Pathology Findings

There were no lesions observed at necropsy in those animals euthanized after the 14-day observation period. The majority of animals that died during the study presented with a serous oral discharge, perianal staining, and intussusception, observations consistent with the actions of a parasympathomimetic agent. Appendix G contains the complete pathology report.

DISCUSSION

The acute oral administration of physostigmine salicylate to Hartley guinea pigs produced pronounced toxicological effects. The calculated median lethal dose (MLD) for physostigmine salicylate in this study was 7.08 mg/kg in male and 5.94 mg/kg in female guinea pigs. These MLD values place physostigmine in the highly toxic classification (1-50 mg/kg) of Hodge and Sterner (7).

The toxicity observed following physostigmine administration was consistent with massive cholinergic stimulation following cholinesterase inhibition (8). Toxic signs attributable to excessive muscarinic stimulation included lacrimation, salivation, and diarrhea. The nicotinic effects observed included tremors, irritability, inactivity, and ataxia as the animals became fatigued. These effects were observed primarily in surviving animals since many animals that received the higher doses died without exhibiting the spectrum of toxic signs observed in animals receiving the lower doses.

The two cases of ileocolic intussusceptions observed in animals 87E00239 and 87E00257 at necropsy is probably related to the acetylcholine release that occurs following physostigmine salicylate administration (8). The prolonged toxicity and late death observed in one female (87E00228) administered 6.31 mg/kg physostigmine salicylate was not expected since the half-life of physostigmine is short in all species tested (9). The mechanism for this delayed toxicity cannot be elucidated from this study. If the delayed toxicity is due to a direct pharmacological effect, it will become readily apparent in subchronic toxicity studies scheduled to be conducted in several species.

TABLE 6(a): Mean Body Weights of Male Guinea Pigs Administered Physostigmine Salicylate

Dose Groups (mg/kg)	At Receipt	At Dosing	Day 7	Termination Day 14*
5.62	235.1† ±4.6(8)	303.0 ±7.7(8)	349.6 ±23.5(5)	360.6 ±19.9(5)
6.31	230.1 ±6.2(8)	300.1 ±5.2(8)	335.7 ±20.3(7)	347.0 ±25.1(7)
6.65	228.1 ±7.4(8)	304.5 ±14.7(8)	375.5 ±18.1(4)	391.0 ±20.2(4)
7.08	228.9 ±5.7(8)	304.0 ±7.1(8)	349.3 ±6.5(4)	363.5 ±9.7(4)
7.50	228.9 ±6.3(8)	301.4 ±8.6(8)	359.3 ±21.2(3)	366.3 ±27.7(3)
Vehicle Control	229.4 ±3.2(5)	305.0 ±8.9(5)	371.6 ±10.8(5)	380.2 ±16.1(5)

*Weight after overnight fast.

†Values are mean ± standard error (number of animals) in grams.

TABLE 6(b): Mean Body Weights of Female Guinea Pigs Administered Physostigmine Salicylate

Dose Groups (mg/kg)	At Receipt	At Dosing	Day 7	Termination Day 14*
4.47	235.0† ±8.8(8)	293.8 ±14.2(8)	317.7 ±20.4(6)	313.3 ±26.9(6)
5.62	233.2 ±4.8(16)	287.5 ±8.4(16)	324.8 ±15.4(8)	327.1 ±14.9(8)
6.31	230.1 ±5.6(8)	273.4 ±8.9(8)	328.0 ±9.8(3)	334.3 ±10.5(3)
6.65	235.1 ±6.6(16)	289.7 ±4.4(16)	340.9 ±10.2(7)	349.1 ±10.6(6)
7.08	236.3 ±3.8(8)	287.6 ±3.9(8)	340.7 ±5.7(3)	333.7 ±10.7(3)
7.50	242.9 ±4.3(15)	278.1 ±6.4(15)	329.5 ±16.4(6)	335.2 ±16.0(6)
8.91	252.8 ±8.8(8)	342.4 ±9.3(8)	-	-
Vehicle Control	216.8 ±7.2(5)	276.8 ±6.0(5)	329.4 ±3.6(5)	329.2 ±3.7(5)

*Weight after overnight fast.

†Values are mean ± standard error (number of animals) in grams.

CONCLUSION

Physostigmine salicylate is a highly toxic compound that produces clinical signs associated with cholinergic stimulation. Calculated MLD values after oral administration were 7.08 mg/kg in male and 5.94 mg/kg in female Hartley guinea pigs.

REFERENCES

1. Kluwe WM, Chinn JC, Feder P, Olson C, Joiner R. Efficacy of pyridostigmine pretreatment against acute soman intoxication in a primate model (Paper No. IX-1). In: Proceedings of the sixth medical chemical defense bioscience review. Columbia, MD (4-6 Aug) 1987:227-234.

2. Leadbeter L, Inns RH, Rylands JM. Treatment of poisoning by soman. Fundam Appl Toxicol 1985; 5:S225-S231.

3. Harris LW, McDonough JH, Sticher DL, Lennox WJ. Protection against both lethal and behavioral effects of soman. Drug Chem Toxicol 1984; 7:605-624.

4. Karczmar AG. History of the research with anticholinesterase agents. In: International Encyclopedia of Pharmacology and Therapeutics, 1970 (Section 13) 1:1-8.

5. Acute oral toxicity study (ALD and LD_{50}). LAIR Standard Operating Procedure OP-STX-36, Letterman Army Institute of Research, Presidio of San Francisco, CA, 15 June 1984.

6. Finney DJ. Probit analysis. 3rd ed. Cambridge: Cambridge University Press, 1981:20-80.

7. Hodge HC, Sterner JH. Tabulation of toxicity classes. American Industrial Hygiene Association Quarterly 1943; 10:93-96.

8. Taylor P. Anticholinesterase agents. In: Gilman AG, Goodman LE, Rall TW, Murad F, eds. The pharmacological basis of therapeutics. 7th ed. New York: Macmillan Publishing Co, Inc, 1985; 110-129.

9. Somani SM, Khaligue A. Pharmacokinetic and pharmacodynamic of physostigmine in the rat after intravenous administration. Drug Metab Dispos 1987;15:627-633.

APPENDICES

APPENDIX A: Chemical Data

Chemical Name: Physostigmine salicylate

Other Names: Eserine salicylate; Physostigmine, 2-hydroxybenzoate; 1, 2, 3, 3a, 8, 8a-Hexahydro-1, 3a, 8-trimethylpyrrolo[2,3-b]indol-5-ol methylcarbamate (ester), (3aS-cis)-, mono (2-hydroxybenzoate) (salt)

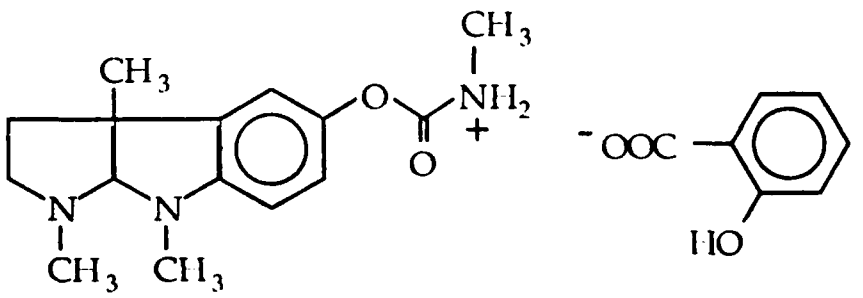
Lot Number: BL25591

Chemical Abstracts Service Registry Number: 57-64-7

LAIR Code: TW73

WRAIR Code: WR 6570AM

Chemical Structure:



Molecular Formula: $C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$

Molecular Weight: 413.47

Analytical Data:

The test compound was analyzed by the sponsors and the identity confirmed by UV and IR spectroscopy, high pressure liquid chromatography, mass spectrometry and elemental analysis.[1] Based on HPLC analysis of this test compound in comparison with the USP physostigmine salicylate reference standard, lot BL25591 contains 66.7% (100.1% of theory) physostigmine base and 33.7% (100.8% of theory) salicylic acid or 100.4% physostigmine salicylate.[1]

HPLC analysis of physostigmine salicylate in this lab was performed using a Hewlett-Packard 1090 HPLC system equipped with a diode array detector. The compound was chromatographed under the following conditions: silica

APPENDIX A (cont.): Chemical Data

column (4.6 x 100 mm, Brownlee Labs, Inc.); mobile phase, 15% acetonitrile/buffer (0.01M Na_2HPO_4 with 0.0025M tetramethylammonium chloride); flow rate, 1.5 ml/min; wavelength monitored, 210 nm. The compound eluted as two peaks with retention times of 0.9 min (salicylic acid), and 3.9 min (physostigmine).[2]

IR (KBr): 3320(broad), 2964, 2325, 1744, 1629, 1594, 1485, 1460, 1383, 1326, 1245, 1203, 1184, 1151, 1140, 1087, 1006, 993, 944, 860, 807, 754, 704, 667, 382 cm^{-1}.[3] The IR spectrum was identical to that provided by the sponsors[1].

Source: Bill Ellis
Division of Experimental Therapeutics
Walter Reed Army Institute of Research
Washington, DC
Requested by LTC Jurgen von Bredow, PhD, MSC

---

[1]Masamori E, Benitez A, and Lim P. Assay of physostigmine salicylate, WR-6570AM, BL25591. Menlo Park, CA: SRI International, 4 November 1986; Report no. 553.

[2]Wheeler CR. Toxicity testing of antidotes of chemical warfare agents. Laboratory notebook #85-12-024.1, pp 2-11. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[3]Wheeler CR. Toxicity testing of antidotes of chemical warfare agents. Laboratory notebook #85-12-024.3, pp 10-11. Letterman Army Institute of Research, Presidio of San Francisco, CA.

APPENDIX A (cont.): Chemical Data

Analysis of Physostigmine Salicylate Dosing Solutions

The concentration of physostigmine salicylate in dosing solutions was determined by UV absorbance at 298 nm. Each solution was diluted and the absorbance measured by a Hitachi 110A Spectrophotometer. Using an extinction coefficient of 6222 L/moles·cm the concentration of physostigmine salicylate was calculated. The concentrations of dosing solutions for GLP study number 87008 are presented below:

Date Sample Prepared	Date Sample Analyzed	Concentration (mg/ml) Target	Actual	% Target Conc.
20 July 87	20 July 87*	0.562	0.546	97.1
20 July 87	20 July 87	0.631	0.627	99.4
20 July 87	22 July 87+	0.631	0.611	96.8
22 July 87	22 July 87	0.665	0.654	98.3
22 July 87	22 July 87	0.708	0.694	98.0
22 July 87	22 July 87	0.750	0.737	98.3
8 Sep 87	8 Sep 87†	0.750	0.747	99.6
8 Sep 87	8 Sep 87	0.665	0.654	98.3
8 Sep 87	8 Sep 87	0.562	0.554	98.6
15 Sep 87	15 Sep 87§	0.891	0.863	96.9

*Wheeler CR. Toxicity testing of antidotes for chemical warfare agents. Laboratory Notebook #85-12-024, p 67. Letterman Army Institute of Research, Presidio of San Francisco, CA.

+Wheeler CR. Toxicity testing of antidotes for chemical warfare agents. Laboratory Notebook #85-12-024.3, p 20. Letterman Army Institute of Research, Presidio of San Francisco, CA.

†Wheeler CR. Toxicity testing of antidotes for chemical warfare agents. Laboratory Notebook #85-12-024.1, p 66. Letterman Army Institute of Research, Presidio of San Francisco, CA.

§*Ibid.* p 67.

APPENDIX B: Animal Data

Species: *Cavia porcellus*

Strain: Hartley

Source: Charles River Laboratories, Inc.
Kingston, NY

Sex: Male and female

Date of birth: Male: 19 June 1987
Female: 19 June and 5 August 1987

Method of randomization: Weight bias, stratified animal allocation (Beckman TOXSYS® Animal Allocation Program, SOP OP-ISG-24)

Animals in each group: 8 male and 16 female animals in Groups 1, 3, and 5(except 15 females in Group 5); 8 male and 8 female animals in Groups 2 and 4; 8 females in Groups 7 and 8; 5 male and 5 female animals for the vehicle control (Group 6).

Condition of animals at start of study: Normal

Body weight range at dosing: 210 - 384 g

Identification procedures: Ear Tag.

Pretest conditioning: Quarantine/acclimation
Phase I: 8 - 20 July 1987
Phase II: 28 August - 7 September 1987

Justification: The guinea pig has proven to be a sensitive and reliable animal model for lethal dose determinations.

APPENDIX C: Historical Listing of Study Events

Date	Event
7 Jul 87	Received 57 male and 56 female Hartley guinea pigs. Animals were sexed and individually caged (Phase I).
8 Jul 87	Four animals (2 male and 2 female) were submitted for necropsy quality control. All animals were checked for physical condition, weighed, and given ear tags.
8-20 Jul 87	Animals were observed daily.
13 Jul 87	Animals were weighed and randomized into dose groups.
20 Jul 87	Animals (Groups 1 and 2) were weighed and removed from quarantine. Food was removed by 1800
21 Jul 87	Animals (Groups 1 and 2) were weighed and dosed at approximately 0900. Observations were conducted at 1, 2, and 4 hours after dosing. Food was re-introduced 2-4 hours after dosing.
22 Jul 87	Animals (Groups 3 - 6) were weighed and removed from quarantine. Food was removed by 1800
23 Jul 87	Animals (Groups 3 - 6) were weighed and dosed at approximately 0900. Observations were conducted at 1, 2, and 4 hours after dosing. Food was re-introduced 2-4 hours after dosing.
22 Jul - 4 Aug 87	Animals were observed for clinical signs in AM and PM (Groups 1 and 2).
24 Jul - 6 Aug 87	Animals were observed for clinical signs in AM and PM (Groups 3 - 6).
28 Jul 87	Animals were weighed (Groups 1 and 2).
30 Jul 87	Animals were weighed (Groups 3 - 6).
3 Aug 87	Food was removed by 1800 (Groups 1 and 2).

APPENDIX C (cont.): Historical Listing of Study Events

Date	Event
4 Aug 87	Animals (Groups 1 and 2) were weighed and observed for clinical signs. Animals were delivered to Necropsy Suite.
5 Aug 87	Food was removed by 1800 (Groups 3 - 6).
6 Aug 87	Animals (Groups 3 - 6) were weighed and observed for clinical signs. Animals were delivered to Necropsy Suite.
27 Aug 87	Phase II animals (females) arrived and were sexed, observed for illness, and individually caged. Forty-one females were assigned to the study.
28 Aug 87	Animals were tagged and weighed, and one female QC animal was submitted to necropsy.
28 Aug - 7 Sep 87	Animals were observed daily while under quarantine.
31 Aug 87	Animals were weighed and randomized into dose groups.
4 Sep 87	All animals were weighed and removed from quarantine.
7 Sep 87	Food was removed by 1800 (Groups 1, 3, and 5).
8 Sep 87	Animals (Groups 1, 3, and 5) were weighed and dosed at approximately 0900. Observations were conducted 1, 2, and 4 hours after dosing.
9-21 Sep 87	Animals (Groups 1, 3, and 5) were observed for clinical signs in AM and PM
9 Sep 87	Food was removed by 1800 (Group 7).
10 Sep 87	Animals (Group 7) were weighed and dosed at approximately 0900. Observations were conducted 1, 2, and 4 hours after dosing.
11-23 Sep 87	Animals (Group 7) were observed for clinical signs in AM and PM.

APPENDIX C (cont.): Historical Listing of Study Events

Date	Event
14 Sep 87	Food (Group 8) was removed by 1800.
15 Sep 87	Animals (Groups 1, 3, and 5) were weighed.
15 Sep 87	Animals (Group 8) were weighed and dosed at approximately 0900. Observations were conducted 1, 2, and 4 hours after dosing. All died and were submitted to the Necropsy Suite on 15 Sep 87, except for # 240 and 248 which were sent to necropsy on 16 Sep 87.
17 Sep 87	Animals (Group 7) were weighed.
21 Sep 87	Food (Groups 1, 3, and 5) was removed by 1800.
22 Sep 87	Animals (Groups 1, 3, and 5) were weighed and observed for clinical signs at approximately 0730. Animals were delivered to Necropsy Suite.
23 Sep 87	Food (Group 7) was removed by 1800.
24 Sep 87	Animals (Group 7) were weighed and observed for clinical signs at approximately 0730. Animals were delivered to Necropsy Suite.

APPENDIX D: Cumulative Mortality Data (Death/Group)
(8 Animals Per Group)

Dose mg/kg	Time After Dosing: Hours 1	2	4	12	Days 1	2	3	4	5	6	7	8-14
Males												
5.62	0	0	1	3	3	3	3	3	3	3	3	3
6.31	0	0	0	0	0	1	1	1	1	1	1	1
6.65	0	0	1	3	4	4	4	4	4	4	4	4
7.08	0	0	2	3	4	4	4	4	4	4	4	4
7.50	0	0	0	3	3	5	5	5	5	5	5	5
0*	0	0	0		0	0	0	0	0	0	0	0
Females												
4.47	0	0	0	1	1	2	2	2	2	2	2	2
5.62†	1	1	3	7	7	8	8	8	8	8	8	8
6.31	1	1	2	2	4	4	4	5	5	5	5	5
6.65†	2	2	6	9	9	9	9	9	9	9	9	9
7.08	0	0	0	5	5	5	5	5	5	5	5	5
7.50$	0	0	4	7	8	9	9	9	9	9	9	9
8.91	0	0	4	8	8	8	8	8	8	8	8	8
0*	0	0	0		0	0	0	0	0	0	0	0
TOTAL	4	4	23	51	56	62	62	63	63	63	63	63

* 5 animals per group
† 16 animals per group
$ 15 animals per group

APPENDIX E: INDIVIDUAL ANIMAL HISTORIES

MALE: 5.62 mg/kg Physostigmine salicylate

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87E00116	Stain, Brown, Perianal	July 21	Slight
	Increased Salivation	July 21	Slight
87E00124	Increased Salivation	July 21	Slight
	Death	July 23	7.3 h
87E00128	Normal	N/A	N/A
87E00133	Stain, Yellow, Perianal	August 1	Slight
87E00140	Death	July 21	4 h
87E00147	Stain, Brown, Perianal	July 21-31	
		August 2,3	Moderate
87E00151	Death	July 22	30 h
87E00152	Diarrhea	August 2,3	Present

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 6.31 mg/kg Physostigmine salicylate

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87E00115	Increased Salivation	July 21	Present
	Death	July 23	43.2 h
87E00132	Increased Salivation	July 21	Present
87E00137	Normal	N/A	N/A
87E00146	Tarry Feces	July 27,28	Present
87E00156	Stain, Brown, Perianal	July 21-30	Slight
	Inactive	July 25-30	Slight
	Rough Coat	July 26-Aug 3	Present
	Diarrhea	July 29-Aug 3	Present
	Emaciated	Aug 1-3	Moderate
87E00163	Normal	N/A	N/A
87E00167	Normal	N/A	N/A
87E00170	Stain, Brown, Perianal	July 21-25	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 6.65 mg/kg Physostigmine salicylate

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87E00118	Normal	N/A	N/A
87E00120	Death	July 23	6.6 h
87E00136	Normal	N/A	N/A
87E00143	Normal	N/A	N/A
87E00144	Death	July 24	24 h
87E00149	Stain, Brown, Perianal Death	July 23 July 23	Moderate 4.0 h
87E00168	Increased Salivation	July 23	Present
87E00171	Increased Salivation Death	July 23 July 23	Present 5.5 h

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 7.08 mg/kg Physostigmine salicylate

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87E00122	Death	July 23	3.6 h
87E00123	Death	July 23	3.6 h
87E00127	Stain, Brown, Perianal	July 24	Slight
	Stain, Yellow, Perianal	July 25-30	Slight
87E00131	Death	July 23	6.6 h
87E00138	Diarrhea	August 2,3	Present
87E00142	Normal	N/A	N/A
87E00159	Death	July 24	24 h
87E00165	Stain, Brown, Perianal	July 23	Slight
	Stain, Yellow, Perianal	July 24-Aug 5	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 7.50 mg/kg Physostigmine salicylate

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87E00117	Normal	N/A	N/A
87E00119	Normal	N/A	N/A
87E00141	Increased Salivation	July 23	Present
	Death	July 23	6.2 h
87E00150	Increased Salivation	July 23	Present
	Inactive	July 23	Slight
	Death	July 23	4.7 h
87E00153	Tremors	July 24	Moderate
	Inactive	July 24	Moderate
	Rough Coat	July 24	Moderate
	Death	July 25	48 h
87E00154	Inactive	July 23	Slight
	Diarrhea	August 5	Present
87E00157	Death	July 25	48 h
87E00166	Stain, Yellow, Perianal	July 23	Slight
	Death	July 23	5.6 h

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: Vehicle Control

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87E00121	Normal	N/A	N/A
87E00125	Normal	N/A	N/A
87E00135	Stain, Brown, Perianal Diarrhea	July 23 Aug 4,5	Slight Present
87E00155	Normal	N/A	N/A
87E00158	Normal	N/A	N/A

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 5.62 mg/kg Physostigmine salicylate

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87E00172	Stain, Brown, Perianal	July 21-31	Slight
	Increased Salivation	July 21	Moderate
	Diarrhea	July 27-31	Present
87E00177	Normal	N/A	N/A
87E00186	Death	July 21	0.1 h
87E00201	Normal	N/A	N/A
87E00211	Stain, Brown, Perianal	July 21	Slight
	Increased Salivation	July 21	Slight
	Death	July 23	47 h
87E00219	Normal	N/A	N/A
87E00226	Normal	N/A	N/A
87E00227	Stain, Brown, Perianal	July 21	Slight
	Death	July 21	4.5 h

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 5.62 mg/kg Physostigmine salicylate (cont.)

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87E00234	Increased Salivation	Sep 8	Slight
87E00237	Increased Salivation	Sep 8	Slight
	Death	Sep 8	7.1 h
87E00242	Increased Salivation	Sep 8	Slight
	Death	Sep 8	6.4 h
87E00249	Stain, Yellow, Perianal	Sep 19-21	Slight
87E00255	Death	Sep 8	3.7 h
87E00256	Diarrhea	Sep 8	Slight
	Death	Sep 8	3.6 h
87E00261	Diarrhea	Sep 9,15-21	Moderate
	Prolapsed Uterus	Sep 10	Moderate
	Uterus, Necrosis	Sep 10	Slight
	Uterus, Sutured	Sep 10	Present
	Drainage, Brown, Vulva	Sep 12,13	Slight
	Drainage, Yellow, Vulva	Sep 17	Moderate
	Material, Brown, Abdomen	Sep 19,20	Moderate
	Material, Brown, Hind Legs	Sep 19,20	Moderate
	Rough Coat	Sep 19-21	Moderate
87E00266	Death	Sep 8	6.2 h

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 6.31 mg/kg Physostigmine salicylate

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87E00188	Stain, Brown, Perianal	July 21	Slight
	Death	July 21	4.0 h
87E00193	Stain, Brown, Perianal	July 21	Slight
	Increased Salivation	July 21	Present
	Death	July 22	18 h
87E00195	Normal	N/A	N/A
87E00202	Death	July 21	0.1 h
87E00203	Death	July 22	24 h
87E00222	Stain, Brown, Perianal	July 21-Aug 3	Moderate
	Diarrhea	July 29	Present
87E00224	Stain, Brown, Perianal	July 21	Slight
87E00228	Increased Salivation	July 21	Present
	Inactive	July 23,24	Moderate
	Emaciated	July 24	Present
	Rough Coat	July 24	Present
	Death	July 25	4 days

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 6.65 mg/kg Physostigmine salicylate

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87E00180	Tremors	July 23	Slight
	Increased Salivation	July 23	Present
	Death	July 23	5.4 h
87E00183	Death	July 23	3.8 h
87E00184	Not Dosed	N/A	N/A
87E00185	Increased Salivation	July 23	Present
	Death	July 23	5.4 h
87E00189	Death	July 23	0.03 h
87E00191	Death	July 23	0.02 h
87E00197	Death	July 23	6.3 h
87E00213	Normal	N/A	N/A
87E00221	Death	July 23	2.9 h

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 6.65 mg/kg Physostigmine salicylate (cont.)

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87E00231	Increased Salivation	Sep 8	Slight
87E00233	Death	Sep 8	2.6 h
87E00241	Hunched Posture	Sep 8	Present
	Stain, Yellow, Abdomen	Sep 9-21	Moderate
87E00251	Increased Salivation	Sep 8	Slight
	Rough Coat	Sep 9	Slight
87E00262	Normal	N/A	N/A
87E00265	Ataxia	Sep 8	Marked
	Increased Salivation	Sep 8	Moderate
	Death	Sep 8	2.4 h
87E00267	Increased Salivation	Sep 8	Slight
87E00269	Increased Salivation	Sep 8	Slight
	Rough Coat	Sep 9	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 7.08 mg/kg Physostigmine salicylate

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87E00173	Increased Salivation	July 23	Present
87E00174	Increased Salivation	July 23	Present
	Death	July 23	5.0 h
87E00175	Death	July 23	6.4 h
87E00178	Increased Salivation	July 23	Present
	Death	July 23	4.9 h
87E00194	Death	July 23	4.9 h
87E00206	Normal	N/A	N/A
87E00218	Increased Salivation	July 23	Slight
	Death	July 23	4.8 h
87E00220	Increased Salivation	July 23	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 7.50 mg/kg Physostigmine salicylate

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87E00198	Normal	N/A	N/A
87E00199	Tremors	July 23	Slight
	Death	July 23	4.6 h
87E00204	Stain, Yellow, Perianal	July 23, Aug 5	Slight
	Increased Salivation	July 23	Present
	Stain, Brown, Perianal	Aug 1,2	Moderate
87E00205	Stain, Yellow, Perianal	July 23	Slight
	Death	July 23	4.6 h
87E00207	Death	July 24	24 h
87E00212	Stain, Brown, Perianal	July 23	Slight
87E00216	Normal	N/A	N/A
87E00221	To Group 3	N/A	N/A

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 7.50 mg/kg Physostigmine salicylate (cont.)

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87E00229	Inactive	Sep 8	Moderate
	Ataxia	Sep 8	Moderate
	Death	Sep 8	2.4 h
87E00239	Paralysis, Hind Limb	Sep 9	Marked
	Moribund	Sep 9	Present
	Death	Sep 10	48 h
87E00244	Increased Salivation	Sep 8	Moderate
87E00247	Increased Salivation	Sep 8	Slight
	Ataxia	Sep 8	Slight
	Death	Sep 8	2.5 h
87E00250	Increased Salivation	Sep 8	Moderate
	Lacrimation	Sep 8	Slight
	Death	Sep 8	4.6 h
87E00258	Increased Salivation	Sep 8	Moderate
	Ataxia	Sep 8	Slight
	Death	Sep 8	2.4 h
87E00259	Increased Salivation	Sep 8	Slight
	Ataxia	Sep 8	Moderate
	Tremors	Sep 8	Slight
	Death	Sep 8	2.3 h
87E00263	Increased Salivation	Sep 8	Slight
	Stain, Yellow, Abdomen	Sep 16-21	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: Vehicle Controls

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87E00176	Stain, Brown, Perianal Diarrhea	July 23 July 29, Aug 1-3	Slight Present
87E00182	Normal	N/A	N/A
87E00200	Stain, Brown, Perianal Diarrhea	July 23 Aug 2,3	Slight Present
87E00215	Normal	N/A	N/A
87E00225	Normal	N/A	N/A

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 4.47 mg/kg Physostigmine salicylate

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87E00230	Increased Salivation	Sep 10	Slight
87E00236	Increased Salivation	Sep 10	Moderate
	Death	Sep 10	6.3 h
87E00245	Increased Salivation	Sep 10	Moderate
	Stain, Brown, Perianal	Sep 12,13	Moderate
	Stain, Brown, Abdomen	Sep 16,17	Slight
	Stain, Yellow, Abdomen	Sep 19-21,23	Slight
87E00252	Increased Salivation	Sep 10	Slight
	Stain, Brown, Perianal	Sep 16-23	Marked
87E00253	Increased Salivation	Sep 10	Slight
	Pallor	Sep 12-16	Moderate
	Inactive	Sep 14-16	Moderate
	Material, Brown, Fr. Leg	Sep 20-23	Moderate
	Diarrhea	Sep 21-23	Present
87E00257	Increased Salivation	Sep 10	Slight
	Loss of Equilibrium	Sep 11	Moderate
	Inactive	Sep 11	Moderate
	Pale Skin Color	Sep 11	Present
	Death	Sep 12	48 h
87E00260	Normal	N/A	N/A
87E00264	Increased Salivation	Sep 10	Slight
	Stain, Brown, Abdomen	Sep 12-17	Moderate
	Stain, Yellow, Abdomen	Sep 19-23	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 8.91 mg/kg Physostigmine salicylate

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87E00235	Increased Salivation	Sep 15	Slight
	Death	Sep 15	4.0 h
87E00238	Death	Sep 15	6.9 h
87E00240	Increased Salivation	Sep 15	Slight
	Death	Sep 15	7.1 h
87E00243	Increased Salivation	Sep 15	Moderate
	Death	Sep 15	3.8 h
87E00246	Increased Salivation	Sep 15	Slight
	Death	Sep 15	6.7 h
87E00248	Increased Salivation	Sep 15	Slight
	Death	Sep 15	6.7 h
87E00254	Increased Salivation	Sep 15	Moderate
	Ataxia	Sep 15	Moderate
	Inactive	Sep 15	Moderate
	Death	Sep 15	2.6 h
87E00268	Death	Sep 15	3.7 h

APPENDIX F: Individual Body Weights

MALES 5.62 mg/kg - Group 1

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14*
87E00116	230†	300	381	384
87E00124	258	335	-	-
87E00128	244	330	377	389
87E00133	237	297	375	387
87E00140	222	266	-	-
87E00147	239	308	257	284
87E00151	216	293	-	-
87E00152	235	295	358	359
Mean	235.1	303.0	349.6	360.6
Standard Deviation	13.0	21.9	52.5	44.5
Std. Error of Mean	4.6	7.7	23.5	19.9

* Weights after an overnight fast.
† Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

MALES 6.31 mg/kg - Group 2

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14*
87E00115	235†	289	-	-
87E00132	223	292	357	371
87E00137	236	310	375	387
87E00146	199	303	375	391
87E00156	235	291	233	206
87E00163	216	279	339	361
87E00167	258	322	295	325
87E00170	239	315	376	388
Mean	230.1	300.1	335.7	347.0
Standard Deviation	17.6	14.8	53.8	66.3
Std. Error of Mean	6.2	5.2	20.3	25.1

* Weights after an overnight fast.
† Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

MALES 6.65 mg/kg - Group 3

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14*
87E00118	238†	305	341	351
87E00120	252	324	-	-
87E00136	233	306	351	362
87E00143	233	351	419	431
87E00144	196	303	-	-
87E00149	195	210	-	-
87E00168	240	329	391	420
87E00171	238	308	-	-
Mean	228.1	304.5	375.5	391.0
Standard Deviation	21.0	41.6	36.2	40.3
Std. Error of Mean	7.4	14.7	18.1	20.2

* Weights after an overnight fast.
† Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

MALES 7.08 mg/kg - Group 4

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14*
87E00122	220†	298	-	-
87E00123	252	339	-	-
87E00127	230	298	351	368
87E00131	236	321	-	-
87E00138	218	304	367	389
87E00142	202	289	341	351
87E00159	247	310	-	-
87E00165	226	273	338	346
Mean	228.9	304.0	349.3	363.5
Standard Deviation	16.2	20.0	13.0	19.4
Std. Error of Mean	5.7	7.1	6.5	9.7

* Weights after an overnight fast.
† Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

MALES 7.50 mg/kg - Group 5

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14*
87E00117	236†	302	355	374
87E00119	248	327	398	410
87E00141	252	306	-	-
87E00150	214	330	-	-
87E00153	226	313	-	-
87E00154	218	269	325	315
87E00157	200	265	-	-
87E00166	237	301	-	-
Mean	228.9	301.4	359.3	366.3
Standard Deviation	17.7	24.3	36.7	48.0
Std. Error of Mean	6.3	8.6	21.2	27.7

* Weights after an overnight fast.
† Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

FEMALES 5.62 mg/kg - Group 1

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14*
87E00172	244†	279	333	338
87E00177	233	262	317	322
87E00186	216	280	-	-
87E00201	209	263	324	303
87E00211	248	386	-	-
87E00219	238	299	352	370
87E00226	226	266	314	309
87E00227	229	282	-	-
87E00234	211	308	369	372
87E00237	259	312	-	-
87E00242	254	276	-	-
87E00249	209	278	360	357
87E00255	237	294	-	-
87E00256	233	263	-	-
87E00261	211	238	229	246
87E00266	273	314	-	-
Mean	233.2	287.5	324.8	327.1
Standard Deviation	19.3	33.4	43.7	42.0
Std. Error of Mean	4.8	8.4	15.4	14.9

*Weights after an overnight fast.
†Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

FEMALES 6.31 mg/kg - Group 2

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14*
87E00188	222†	252	-	-
87E00193	217	263	-	-
87E00195	231	273	323	337
87E00202	265	326	-	-
87E00203	220	255	-	-
87E00222	229	260	314	315
87E00224	239	295	347	351
87E00228	218	263	-	-
Mean	230.1	273.4	328.0	334.3
Standard Deviation	16.0	25.1	17.1	18.1
Std. Error of Mean	5.6	8.9	9.8	10.5

* Weights after an overnight fast.
† Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

FEMALES 6.65 mg/kg - Group 3

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14*
87E00180	226†	283	-	-
87E00183	233	299	-	-
87E00185	205	272	-	-
87E00189	225	304	-	-
87E00191	235	301	-	-
87E00197	258	309	-	-
87E00213	235	289	331	338
87E00221	191	277	-	-
87E00231	237	294	358	377
87E00233	198	284	-	-
87E00241	296	324	384	385
87E00251	273	300	341	358
87E00262	249	266	336	339
87E00265	222	298	-	-
87E00267	246	284	341	346
87E00269	233	252	295	301
Mean	235.1	289.8	340.9	349.1
Standard Deviation	26.6	17.8	27.0	28.0
Std. Error of Mean	6.6	4.4	10.2	10.6

* Weights after an overnight fast.
† Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

FEMALES 7.03 mg/kg - Group 4

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14*
87E00173	237†	289	336	336
87E00174	243	288	-	-
87E00175	232	305	-	-
87E00178	253	299	-	-
87E00194	237	272	-	-
87E00206	235	280	352	351
87E00218	238	291	-	-
87E00220	215	277	334	314
Mean	236.3	287.6	340.7	333.7
Standard Deviation	10.7	11.1	9.9	18.6
Std. Error of Mean	3.8	3.9	5.7	10.7

* Weights after an overnight fast.
† Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

FEMALES 7.50 mg/kg - Group 5

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14*
87E00198	237†	271	345	340
87E00199	241	289	-	-
87E00204	225	244	274	286
87E00205	231	281	-	-
87E00207	241	306	-	-
87E00212	244	238	295	305
87E00216	236	309	383	390
87E00279	225	239	-	-
87E00239	254	313	-	-
87E00244	270	303	356	369
87E00247	277	294	-	-
87E00250	254	267	-	-
87E00258	217	281	-	-
87E00259	236	268	-	-
87E00263	255	268	324	321
Mean	242.9	278.1	329.5	335.2
Standard Deviation	16.6	24.8	40.3	39.2
Std. Error of Mean	4.3	6.4	16.4	16.0

* Weights after an overnight fast.
† Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

VEHICLE CONTROLS - Group 6

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14*
		MALES		
87E00121	223†	291	361	349
87E00125	238	326	386	403
87E00135	233	326	406	431
87E00155	232	299	359	369
87E00158	221	283	346	349
Mean	229.4	305.0	371.6	380.2
Standard Deviation	7.2	20.0	24.1	36.0
Std. Error of Mean	3.2	8.9	10.8	16.1
		FEMALES		
87E00176	225	278	328	326
87E00182	188	265	320	322
87E00200	222	299	336	325
87E00215	226	275	324	330
87E00225	223	267	339	343
Mean	216.8	276.8	329.4	329.2
Standard Deviation	16.2	13.5	8.0	8.2
Std. Error of Mean	7.2	6.0	3.6	3.7

* Weights after an overnight fast.
† Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

FEMALES 4.47 mg/kg - Group 7

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14*
87E00230	208†	277	322	333
87E00236	277	384	-	-
87E00245	241	294	349	359
87E00252	250	292	356	360
87E00253	226	273	225	207
87E00257	208	290	-	-
87E00260	215	244	302	257
87E00264	255	296	352	364
Mean	235.0	293.8	317.7	313.3
Standard Deviation	25.0	40.3	50.0	65.9
Std. Error of Mean	8.8	14.2	20.4	26.9

* Weights after an overnight fast.
† Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

FEMALES 8.91 mg/kg - Group 8

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14*
87E00235	283†	378	-	-
87E00238	225	348	-	-
87E00240	290	359	-	-
87E00243	252	334	-	-
87E00246	232	368	-	-
87E00248	242	299	-	-
87E00254	230	317	-	-
87E00268	268	336	-	-
Mean	252.8	342.4	-	-
Standard Deviation	25.0	26.4	-	-
Std. Error of Mean	8.8	9.3	-	-

* Weights after an overnight fast.
† Weights are given in grams.

APPENDIX G: Pathology Report

Pathology Report GLP 87008

Acute Oral Toxicity

I. Compound: Physostigmine salicylate
Species: Cavia porcellus, Hartley, young adult.

II. Principal Investigator: CPT Denzil F. Frost
Pathologist: MAJ Charles B. Clifford

III. Comment: No gross lesions were observed in animals surviving the fourteen days of the study. Gross observations in cases of unscheduled deaths are compatible with either generalized increased parasympathetic tone (perioral staining and intussusception) or common incidental findings (hepatic necrosis) of little clinical significance in guinea pigs. No evidence of direct tissue damage due to physostigmine salicylate was observed in any animal.

Charles B Clifford

CHARLES B. CLIFFORD, DVM
MAJ, VC
Division of Pathology

12 November 1987/dbj

APPENDIX G (cont.): Pathology Report

ATTACHMENT:

GROUP #1 (5.62 mg/kg) 16 animals, dosed 21 Jul 87, 10 animals sacrificed 4 Aug 87.

ANIMAL ID#	LAIR ACC#	SEX	NECROPSY DATE	DIAGNOSES
87E00116	41444	M	4 Aug 87	No lesions recognized
128	41445	M	4 Aug 87	No lesions recognized
133	41447	M	4 Aug 87	No lesions recognized
147	41450	M	4 Aug 87	No lesions recognized
152	41451	M	4 Aug 87	No lesions recognized
172	41456	F	4 Aug 87	No lesions recognized
177	41457	F	4 Aug 87	No lesions recognized
201	41459	F	4 Aug 87	No lesions recognized
219	41460	F	4 Aug 87	No lesions recognized
226	41463	F	4 Aug 87	No lesions recognized
GROUP #1 Spontaneous deaths, 6 animals				
87E00124	41344	M	21 Jul 87	Serous oral discharge
140	41343	M	21 Jul 87	Serous oral discharge
151	41348	M	22 Jul 87	Brown perioral staining
186	41341	F	21 Jul 87	No lesions recognized
211	42065	F	23 Jul 87	No lesions recognized
227	41342	F	21 Jul 87	Serous oral discharge
GROUP #2 (6.31 mg/kg), 16 animals, dosed 21 Jul 87, 10 animals sacrificed 4 Aug 87.				
87E00132	41446	M	4 Aug 87	No lesions recognized
137	41448	M	4 Aug 87	No lesions recognized
146	41449	M	4 Aug 87	No lesions recognized
156	41452	M	4 Aug 87	No lesions recognized
163	41453	M	4 Aug 87	No lesions recognized
167	41454	M	4 Aug 87	No lesions recognized
170	41455	M	4 Aug 87	No lesions recognized
195	41458	F	4 Aug 87	No lesions recognized
222	41461	F	4 Aug 87	No lesions recognized
224	41462	F	4 Aug 87	No lesions recognized
GROUP #2 Spontaneous deaths, 6 animals				
87E00115	41449	M	23 Jul 87	No lesions recognized
188	41447	F	22 Jul 87	No lesions recognized
193	41345	F	22 Jul 87	Serous oral discharge, mild, focal, hepatic necrosis.
202	41340	F	21 Jul 87	Ingesta present around and in mouth, mild hemopericardium, mild, focal, hepatic necrosis.
203	41346	F	22 Jul 87	No lesions recognized
228	41378	F	25 Jul 87	No lesions recognized

APPENDIX G (cont.): Pathology Report

GROUP #3 (6.65 mg/kg), 24 animals, dosed 23 Jul 87, 5 animals sacrificed 6 Aug 87.

ANIMAL ID#	LAIR ACC#	SEX	NECROPSY DATE	DIAGNOSES
87E00118	41482	M	6 Aug 87	No lesions recognized
136	41488	M	6 Aug 87	No lesions recognized
143	41491	M	6 Aug 87	No lesions recognized
168	41496	M	6 Aug 87	No lesions recognized
213	41505	F	6 Aug 87	No lesions recognized
GROUP #3	Spontaneous deaths, 13 animals, #1			
87E00120	41371	M	23 Jul 87	Serous oral discharge
144	41375	M	24 Jul 87	No lesions recognized
149	41356	M	23 Jul 87	Yellow-brown perioral and perianal staining
171	41368	M	23 Jul 87	No lesions recognized
180	41360	F	23 Jul 87	Serous oral discharge
183	41354	F	23 Jul 87	Serous oral discharge
185	41361	F	23 Jul 87	Serous oral discharge
189	42064	F	23 Jul 87	No lesions recognized
191	42063	F	23 Jul 87	Brown perinasal staining
197	41369	F	23 Jul 87	Ingesta around oral cavity
221	41357	F	23 Jul 87	Ingesta around oral cavity

#1 Additionally, 87F00184 (Accession #41464), a female was not dosed due to excessive weight loss, and was found dead 30 Jul 87. Gross observations included extensive matting of fur with diarrhea, severe dehydration and emaciation.

GROUP #4 (7.08 mg/kg), 9 animals dosed 23 Jul 87. Seven animals sacrificed 6 Aug 87.

87E00127	41486	M	6 Aug 87	No lesions recognized
138	41489	M	6 Aug 87	No lesions recognized
142	41490	M	6 Aug 87	No lesions recognized
165	41495	M	6 Aug 87	No lesions recognized
173	41497	F	6 Aug 87	No lesions recognized
206	41503	F	6 Aug 87	No lesions recognized
220	41508	F	6 Aug 87	No lesions recognized

APPENDIX G (cont.): Pathology Report

GROUP #4 Spontaneous deaths, 9 animals

ANIMAL ID#	LAIR ACC#	SEX	NECROPSY DATE	DIAGNOSES
87E00122	41358	M	23 Jul 87	Serous oral discharge
123	41355	M	23 Jul 87	Serous oral discharge
131	41372	M	23 Jul 87	Serous oral discharge
159	41376	M	24 Jul 87	Serous oral and ocular discharge
174	41362	F	23 Jul 87	Brown perioral staining
175	41374	F	23 Jul 87	Green-brown perioral and perianal staining
178	41364	F	23 Jul 87	Serous oral discharge
194	41367	F	23 Jul 87	Serous oral discharge
218	41363	F	23 Jul 87	Serous oral discharge

GROUP #5 (7.5 mg/kg) 15 animals, dosed 23 Jul 87. 7 animals sacrificed 6 Aug 87.

87E00117	41481	M	6 Aug 87	No lesions recognized
119	41483	M	6 Aug 87	No lesions recognized
154	41492	M	6 Aug 87	No lesions recognized
198	41500	F	6 Aug 87	No lesions recognized
204	41502	F	6 Aug 87	No lesions recognized
212	41504	F	6 Aug 87	No lesions recognized
216	41507	F	6 Aug 87	No lesions recognized

GROUP #5 Spontaneous deaths, 8 animals

87E00141	41373	M	23 Jul 87	Serous oral discharge
150	41366	M	23 Jul 87	No lesions recognized
153	41379	M	25 Jul 87	Multifocal, mild hepatic necrosis
157	41380	M	25 Jul 87	No lesions recognized
166	41370	M	23 Jul 87	Serous oral discharge
199	41365	F	23 Jul 87	Serous oral discharge
205	41359	F	23 Jul 87	Serous oral discharge
207	41377	F	24 Jul 87	No lesions recognized

GROUP #6 (Control Group 0 mg/kg) 10 animals, all sacrificed 6 Aug 87

87E00121	41484	M	6 Aug 87	No lesions recognized
125	41485	M	6 Aug 87	No lesions recognized
135	41487	M	6 Aug 87	No lesions recognized
155	41493	M	6 Aug 87	No lesions recognized
158	41494	M	6 Aug 87	No lesions recognized
176	41498	F	6 Aug 87	No lesions recognized
182	41499	F	6 Aug 87	No lesions recognized
200	41501	F	6 Aug 87	No lesions recognized
215	41506	F	6 Aug 87	No lesions recognized
225	41509	F	6 Aug 87	No lesions recognized

APPENDIX G (cont.): Pathology Report

Additional Animals:

GROUP #1 (5.62 mg/kg), 8 animals, dosed 8 Sep 87. Three animals sacrificed 22 Sep 87.

ANIMAL ID#	LAIR ID#	SEX	DIAGNOSES
87E00234	41748	F	No lesions recognized
249	41749	F	No lesions recognized
261	41750	F	Weight loss, diarrhea

Spontaneous Deaths, 5 animals

237	41711	F	No lesions recognized. Necropsied 9 Sept 1987.
242	41710	F	No lesions recognized. Necropsied 9 Sept 1987.
255	41707	F	No lesions recognized. Necropsied 8 Sept 1987.
256	41708	F	No lesions recognized. Necropsied 8 Sept 1987.
266	41712	F	No lesions recognized. Necropsied 9 Sept 1987.

GROUP #3 (6.65 mg/kg), 8 animals, dosed 8 Sep 87. Six animals sacrificed 22 Sep 87.

87E00231	41742	F	No lesions recognized
241	41743	F	No lesions recognized
251	41744	F	No lesions recognized
262	41745	F	No lesions recognized
267	41746	F	No lesions recognized
269	41747	F	No lesions recognized

Spontaneous Deaths, 2 animals. Necropsied: 8 Sep 87

233	41701	F	No lesions recognized
265	41702	F	No lesions recognized

GROUP #5 (7.5 mg/kg), 8 animals, dosed 8 Sep 87. Three animals sacrificed 22 Sep 87.

87E00244	41740	F	No lesions recognized
250	41709	F	No lesions recognized
263	41741	F	No lesions recognized

Spontaneous Deaths, 5 animals. (Necropsied 8 Sept 87 unless otherwise stated.)

229	41705	F	No lesions recognized.
239	41713	F	10 cm intussusception at ileocolic junction, with necrosis and hemorrhage. Necropsied 10 Sep 87.
247	41704	F	No lesions recognized.
258	41703	F	No lesions recognized.
259	41706	F	No lesions recognized.

APPENDIX G (cont.): Pathology Report

GROUP #7 (4.47 mg/kg), 8 animals, dosed 10 Sep 87. Six animals sacrificed 24 Sep 87.

ANIMAL ID#	LAIR ID#	SEX	DIAGNOSES
87E00230	41755	F	No lesions recognized
245	41756	F	No lesions recognized
252	41757	F	No lesions recognized
253	41758	F	Diarrhea, diminished body fat.
260	41759	F	No lesions recognized
264	41760	F	No lesions recognized
Spontaneous Deaths, 2 animals			
236	41719	F	No lesions recognized. Necropsied 11 Sept 1987.
257	41720	F	Ileocolic intussusception with necrosis of the intussusceptum. Necropsied 14 Sept 1987.

GROUP #8 (8.91 mg/kg), 8 animals, dosed 15 Sep 87. All spontaneous deaths.

87E00235	41724	F	No lesions recognized
238	41726	F	No lesions recognized
240	41728	F	No lesions recognized. Necropsied 16 Sept 1987
243	41723	F	No lesions recognized
246	41725	F	No lesions recognized
248	41727	F	Ileum congestion with marked congestion of Peyer's Patches. Necropsied 16 Sept 1987.
254	41721	F	No lesions recognized.
268	41722	F	No lesions recognized.

OFFICIAL DISTRIBUTION LIST

Commander
US Army Medical Research
& Development Command
ATTN: SGRD-RMS/Mrs. Madigan
Fort Detrick, MD 21701-5012

Defense Technical Information Center
ATTN: DTIC/DDAB (2 copies)
Cameron Station
Alexandria, VA 22304-6145

Office of Under Secretary of Defense
Research and Engineering
ATTN: R&AT (E&LS), Room 3D129
The Pentagon
Washington, DC 20301-3080

DASG-AAFJML
Army/Air Force Joint Medical Library
Offices of the Surgeons General
5109 Leesburg Pike, Room 670
Falls Church, VA 22041-3258

HQ DA (DASG-ZXA)
WASH DC 20310-2300

Commandant
Academy of Health Sciences
US Army
ATTN: HSHA-CDM
Fort Sam Houston, TX 78234-6100

Uniformed Services University of
Health Sciences
Office of Grants Management
4301 Jones Bridge Road
Bethesda, MD 20814-4799

US Army Research Office
ATTN: Chemical and Biological
Sciences Division
PO Box 12211
Research Triangle Park, NC 27709-2211

Director
ATTN: SGRD-UWZ-L
Walter Reed Army Institute of Research
Washington, DC. 20307-5100

Commander
US Army Medical Research Institute
of Infectious Diseases
ATTN: SGRD-ULZ-A
Fort Detrick, MD 21701-5011

Commander
US Army Medical Bioengineering Research
and Development Laboratory
ATTN: SGRD-UBG-M
Fort Detrick, Bldg 568
Frederick, MD 21701-5010

Commander
US Army Medical Bioengineering
Research & Development Laboratory
ATTN: Library
Fort Detrick, Bldg 568
Frederick, MD 21701-5010

Commander
US Army Research Institute
of Environmental Medicine
ATTN: SGRD-UE-RSA
Kansas Street
Natick, MA 01760-5007

Commander
US Army Research Institute of
Surgical Research
Fort Sam Houston, TX 78234-6200

Commander
US Army Research Institute of
Chemical Defense
ATTN: SGRD-UV-AJ
Aberdeen Proving Ground, MD 21010-5425

Commander
US Army Aeromedical Research
Laboratory
Fort Rucker, AL 36362-5000

AIR FORCE Office of Scientific
Research (NL)
Building 410, Room A217
Bolling Air Force Base, DC 20332-6448

USAF School of Aerospace Medicine
Document Section
USAFSAM/TSKD
Brooks Air Force Base, TX 78235-5301

Head, Biological Sciences Division
OFFICE OF NAVAL RESEARCH
800 North Quincy Street
Arlington, VA 22217-5000